

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Tominaga et al.

Art Unit: 1645

Application No.: 10/772,272

Examiner: FORD, VANESSA L

Filing Date: February 6, 2004

Attorney Ref. No.: US-108

For: INOSINE PRODUCING
BACTERIUM BELONGING TO
THE GENUS BACILLUS AND
METHOD FOR PRODUCING
INOSINE

DECLARATION UNDER 37 C.F.R. § 1.132

I, Takayuki ASAHARA, declare as follows:

1. I am an employee of Ajinomoto Co., Inc. located at 1 -1 Suzuki-shi, Kawasaki-ku, Kawasaki-shi, Kanagawa, Japan, which is engaged in the business of research and development of fermentation processes and products, among other things.
2. I belong to the Fermentation and Biotechnology Laboratories of Ajinomoto Co., Inc. and am engaged in research in the fields of fermentation processes of nucleic acids and gene manipulation techniques .
3. I have conducted the experiments described herein and present them as evidence in the above-identified patent application for consideration by the Examiner in support of the applicant's arguments in response to the Office Action dated October 23, 2006 .
4. The data described herein shows that the phenotype of 6-etoxyurine-resistance is not inherent to a purR-disrupted strain but is imparted with a mutagenesis technique as described in Example 2 of the present specification. That is, the purR-disrupted strain disclosed in U.S. Patent 6,284,495 is not resistant to 6 -etoxyurine, and the document does

not anticipate the present invention.

5. Experiments

The etoxypurine-resistance of a wild-type strain, a purR-disrupted strain and an etoxypurine-resistant strain was evaluated.

Specifically, a wild-type *Bacillus subtilis* SB112 strain, a purR-disrupted strain SB112 Δ purR and an etoxypurine-resistant EP-1 strain which had been obtained in Example 2 of the present specification were cultured in normal medium and in etoxypurine-containing medium, respectively, and their etoxypurine-resistance was evaluated.

Pre-culture

Glycerol stock of each of the strains was spread over the LB-agar medium and the strains were cultured at 34 °C overnight. Then, the obtained cells were collected by platinum loop, inoculated into 4ml of LB liquid medium and cultured at 34 °C overnight. After culturing about 14 hours, 1 ml of the culture was centrifuged to collect cells, and the obtained cells were washed twice with the 121+Ade+Trp+Phe medium shown below, and suspended into 1 ml of the 121+Ade+Trp+Phe medium.

Main Culture

Eight microliters of the cell suspension as prepared above were inoculated into 4 ml of the 121+Ade+Trp+Phe medium or the 121+Ade+Trp+Phe medium containing 2000mg/l of etoxypurine, respectively, and the cells were cultured at 34 °C for 30 hours with an isothermal shaker (Advantec).

Composition of the 121 +Ade+Trp+Phe medium

0.12 M Tris-HCl (pH7.5)
80 mM NaCl
20 mM KCl
20 mM NH₄Cl
1 mM MgCl₂
0.2 mM CaCl₂
2.5 mM Na₂SO₄

2 μ M ZnCl₂
0.2 % Glucose
40 μ g/ml FeCl₃•6H₂O
0.1 % Na₃citrate•H₂O
0.1 mM MnCl₂
0.1 % casamino acids
2.5 mM K₂HPO₄
2 μ M vitaminB1
20 mg/L adenine
20 mg/L tryptophan
20 mg/L phenylalanine

6. Results

The results of the growth of each strain in normal medium and etoxypurine-containing medium are shown in Table 1.

Table 1. Relative growth rate after 30-hour culture

strain	121+Ade+Trp+Phe medium	121+Ade+Trp+Phe medium+2000mg/L etoxypurine
SB112	0.215	0.175
SB112 Δ purR	0.225	0.031
EP-1	0.221	0.224

9. I further declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: Takayuki Asahara
Takayuki ASAHARA

Date: March 08, 2007